SOME OBSERVATIONS ON THE REFRACTORY PERIOD OF THE HEART.

by

TA-MING TSO, M.B.,Ch.B.

A Thesis presented to the Medical Faculty of the University of Edinburgh for the degree of Doctor of Medicine.

A.D. 1929.

SOME OBSERVATIONS ON THE REFRACTORY PERIOD OF THE HEART.

INTRODUCTION.

Ever since the introduction of the conception of circus movement in auricular fibrillation and auricular flutter and the introduction of quinidine for their treatment, the refractory period has assumed an increasingly important rôle in understanding the pathology and the therapeutics of many disorders of cardiac contraction. A thorough knowledge of its physiology, therefore, is of great importance. Unfortunately, however, comparatively little has been done in this direction. The object of the present investigation is to study the refractory period of the frog's heart under several experimental conditions and its relation to other phenomena associated with cardiac contraction. The subject matter will be considered under the following headings:-

I. The effect of variations in the diastolic filling on the durations of the refractory period and of the mechanical response.

II. The effect of various ions on the duration of the refractory period and on the mechanical response.

III. The effect of rate of stimulation on the durations of the refractory period and of

the mechanical response.

IV. The relation of the refractory period to other phenomena associated with contraction.

I. The Effect of Diastolic Filling on the Durations of the Refractory Period and of the Mechanical Response.

A number of experiments were performed to determine whether the durations of the refractory period and of the mechanical response were altered by variations in the diastolic filling. Such variations have been shown to influence profoundly the pressure added by contraction (1,2,3,4), and oxygen consumption (5,6), and the heat liberated by the heart (7). In particular, Clark and White (6) have shown that the empty contracting heart uses very little more oxygen than the empty arrested heart, whereas the heart contracting when filled uses four or five times this quantity. The extent of the metabolic change resulting from the contraction process can, therefore, be varied very extensively by simply varying the diastolic filling of the heart.

General Methods.

The isolated ventricle of R. Temporaria was used and the experiments were carried out in winter and spring at room temperature. The temperature varied on different days from 12°C. to 15°C. but remained constant during the course of any experiment.

The Ringer's fluid used had the percentage composition of Nacl 0.65, Kcl. 0.015; $CaCl_2$ anhyd. 0.011; Na_2HPO_4 and NaH_2PO_4 0.05. The phosphate was added from a stock phosphate solution with a pH of 7.5.

The apparatus for perfusing the ventricle, for varying its diastolic filling, and for recording its isochoric response was similar to that described by Clark (8). It is represented diagrammatically in Fig. 1. The heart contracted under isobaric conditions except when measurements were taken, and then isocharic conditions were maintained.

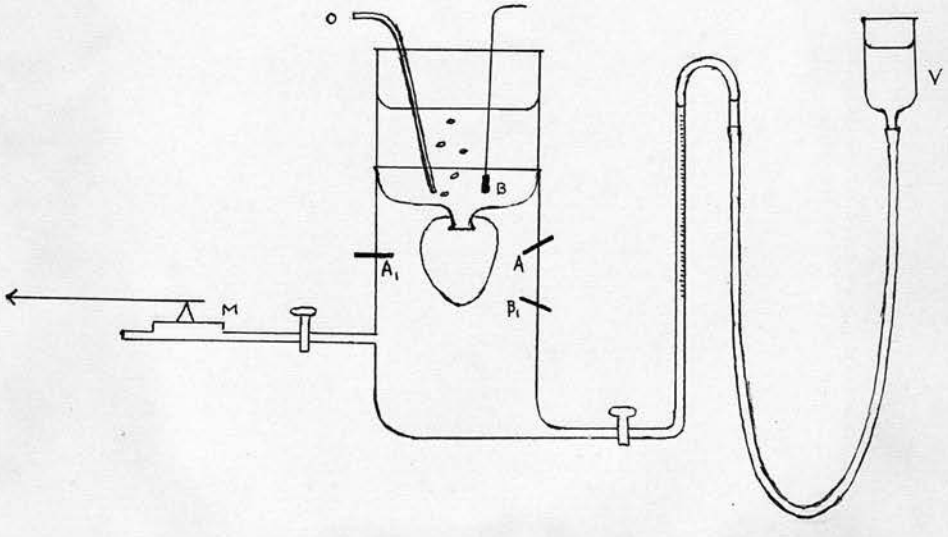


Fig. 1.
M = Rubber Manometer.
V = Variable Fluid Level.
O = Compressed Air.

Arrangements for stimulation of the heart.

A Lewis rotary contact breaker provided with two pairs of cams was used. Each pair of cams was connected with an induction coil, and the wiring was arranged so that the make shock was short-circuited, and only the break shock was allowed to reach the preparation (Fig. 2).

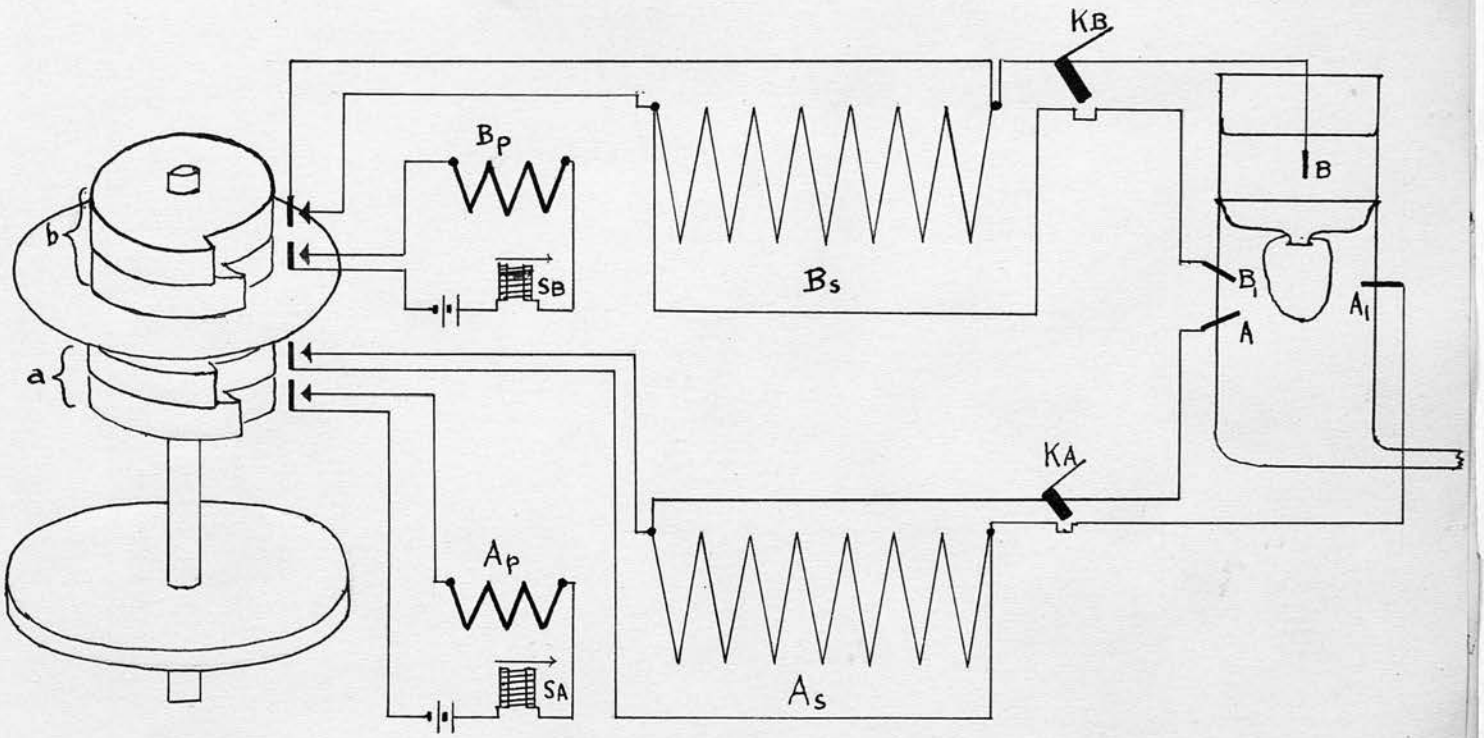


Fig 2.

A_P, A_S = Primary and Secondary coils respectively of Induction Coil A
B_P, B_S = " " " " " " " " B
SA, SB = Signal markers.
KA = Short-circuiting Key in circuit A.
KB = " " " " " B.
A, A_1, B, B_1 = Electrodes.

One coil (A) and one pair of cams (a) was used to produce the initial stimulus, and the fundamental rate of stimulation was usually 18 per minute. This

frequency could be varied by varying the rate of rotation of the contact breaker.

The second coil (B) and the second pair of cams (b) provided the second or extra stimulus. The distance between cams a and b could be varied, and thus the second stimulus could be thrown in at any point in the cardiac cycle. Two signal markers recorded the stimuli on the tracing, and the duration of the interval between the stimuli was measured from these records.

The strength of the stimuli was adjusted by moving the secondary coil of the induction coils, and these were celebrated with a voltimeter, so that the actual strength of the stimulus passing through the preparation was known. The strength of stimulus A was not altered during an experiment and was always well above the threshold, strength, being usually three times that of the threshold strength. The strength of stimulus B was varied according to circumstances.

Determination of the Refractory Period.

The absolute refractory period (A.R.P.) was determined by the usual method of sending in a pair of stimuli and finding out the earliest point in the cardiac cycle at which the second stimulus (stimulus B) was capable of producing a response. For this determination the strength of stimulus B was made about 40 times the threshold strength. In all cases

five pairs of stimuli were passed in succession, and the result was considered positive, if a second contraction was obtained after any or all of these paired stimuli. The duration of the refractory period decreases when the frequency of stimulation is increased and hence once the second stimulus had produced a contraction it continued to do so. The practice of observing the results of five successive pairs of stimuli was a safeguard against errors which will be considered later.

In determining the relative refractory period (R.R.P.), the minimal effective strength of the second stimulus at various points in the cardiac cycle was determined, and a curve of recovery of excitability was thus plotted. The perfusion fluid used was on the alkaline side of neutrality (pH7.5) and a supernormal phase was not usually present. Therefore, the curve of recovery of excitability was usually measured only as far as the end of the relative refractory period.

The tracings obtained were measured with a reading microscope. In some cases tracings were made on smoked celluloid films, and the image was projected on to a screen where it was measured. The results obtained by the two methods were in close agreement. It was found that the tracings could be measured accurately to the nearest 0.05"; hence the error in measurement was about $\pm$ 3 per cent of the duration of

the cardiac cycle.

Sources of Error in the Determination of the Refractory Period.

(a) Changes in the heart produced by stimuli which failed to produce a mechanical response.

Drury and Love (9) in the veratrinised frog's heart first measured the A.R.P. by the usual method, namely by determining the earliest time interval at which a second stimulus produced an electrical response. They then found that a stimulus falling within this period, although producing no electrical response, might still produce a change in the heart, for it might cause a third stimulus put in shortly after it to fail to produce a response. They concluded that the true A.R.P. in the veratrinised heart was shorter than the A.R.P. as measured by the usual method. They did not, however, observe this effect in the normal heart.

In the experiments described here the A.R.P. was not determined according to Drury and Love's method because in the first place, the heart was not abnormal and in the second place, a series of five pairs of stimuli was used to guard against this error. Supposing the above effect was obtained, an extra stimulus sent in between the true and the apparent A.R.P., although apparently ineffective, might shorten the A.R.P. in a subsequent response, since increased

frequency of stimulation shortens the refractory period. This shortening of the A.R.P. would enable subsequent extra stimuli to become effective. On the other hand, if the extra stimulus were sent in before the true A.R.P. was over there would be no shortening of the Refractory period, and hence a second response would not result no matter how often the extra stimulus was repeated. Thus, by always observing the result of a series of five pairs of stimuli this error could be eliminated.

(b) Short circuiting through electrodes.

It was found that the arrangement of the electrodes was a matter of great importance. At first, the electrodes were arranged as in Fig. 3.

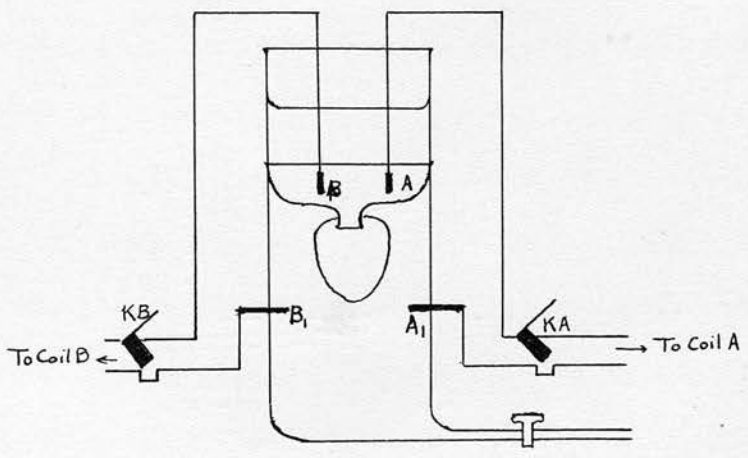


Fig. 3.

This arrangement had to be discarded because when the key (K_B) in circuit B was closed, the current in circuit A instead of passing from A-A_1 (through the ventricle) often passed by the alternative route A-B-B_1-A_1, and hence failed to stimulate the heart. Similarly the current in circuit B passed by corresponding alternative route when the key (K_A) in circuit A was closed. The arrangement of electrodes shown in Fig. 2 was designed to overcome this difficulty.

Under this arrangement, there was only one way for a current in circuit B to complete its course, namely through the ventricle (B-B_1). This ensured uniformity in the strength of stimulus B irrespective of the opening or closing of the key in circuit A. As to stimulus A, the alternative route A-B_1-A_1 always offered greater resistance than the direct route A-A_1 no matter whether the key in circuit B was open or closed, and hence it was most unlikely that the current would travel by the alternative route. Even were this to occur, the resulting variation in the strength of stimulus A would not be of great importance because firstly, stimulus A was always well above the threshold strength and secondly, it has been shown by Hermann and Umrath (10) that the strength of the initial stimulus does not influence the duration of the refractory period of the heart. On the other hand, Kupelweiser (10a) concluded from his studies

that the strength of the initial stimulus did have an influence on the duration of the refractory period. The general trend of opinions among physiologists, however, is to favour the conclusion of the former workers.

(c) Inadequate perfusion.

Boesch (11) and Carter and Dieudaide (12) have shown respectively that lack of oxygen and acidity have a great influence on the duration of the refractory period. When the heart is inadequately perfused it suffers from lack of oxygen, and acid products accumulate. Therefore, to avoid this error, isochoric conditions were maintained only for 6 or 8 successive contractions.

(d) Age of the preparation.

Prolonged washing of the frog's heart with Ringer's fluid produces a hypodynamic state (Clark (13)). Junkmann (14) showed that the refractory of the frog's heart and the duration of mechanical response shortened considerably after prolonged perfusion. This fact was confirmed by the writer . The curve of recovery of excitability of a heart before and after two hours' perfusion with Ringer's fluid is shown in Fig. 4. This shows a shortening of both the A.R.P. and the R.R.P. This fact made it necessary to do frequent controls when experiments of long duration were made.

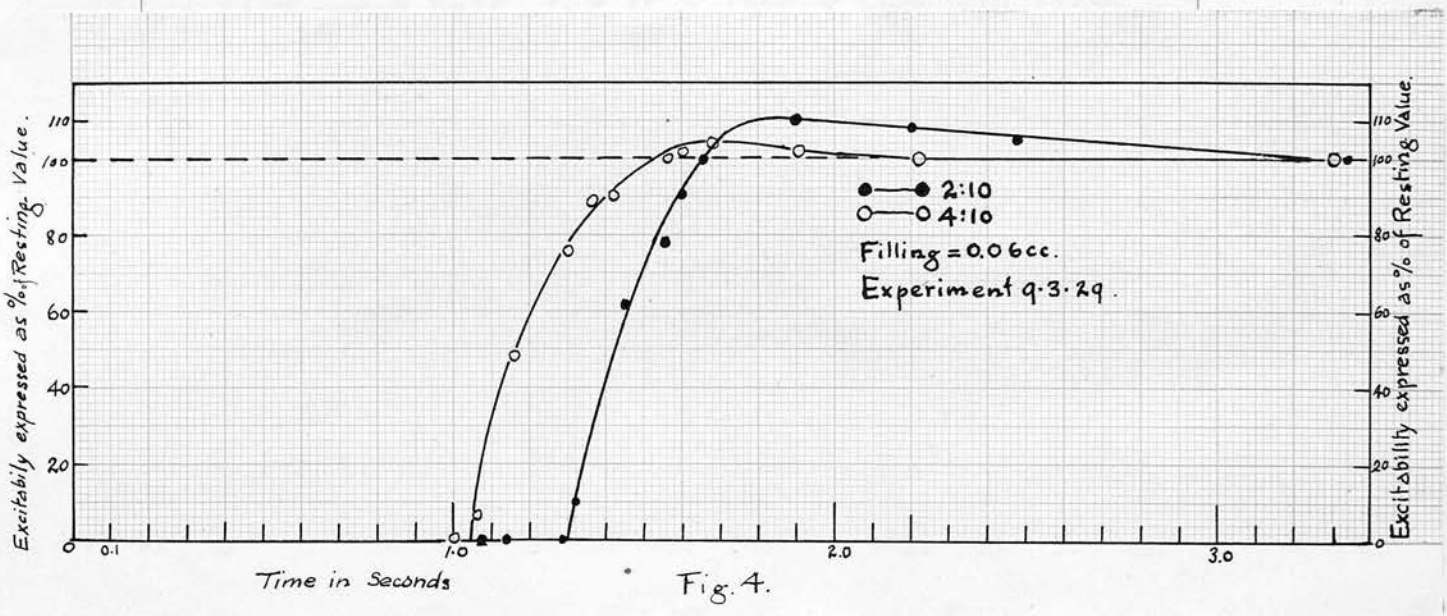


Fig. 4.

(e) Spontaneous contraction of the ventricle.

This frequently interfered with the experiments. It frequently happened that a ventricle which was at first arrested started spontaneous contractions after one or two hours' isolation. In such cases it was usually necessary to stop the experiment because the fundamental rate of contraction and with it the duration of the refractory period was thereby changed.

A. Effect of Diastolic Filling on the Refractory Period.

Experience showed that it was necessary to carry through a set of experiments in less than an hour and a half, for if a longer time was taken the shortening of the refractory period due to the development of the hypodynamic state introduced a large error. For this reason, in some experiments either the A.R.P. or the end of the R.R.P. was determined for a series of different initial fillings. Such determinations could be carried out quickly and hence there was no chance of error due to change in the condition of the preparation. Typical results are recorded in Table 1, p. In Table 1 each determination of the A.R.P. is indicated by two numbers with the sign - or + following them. The former is the longest time interval between stimuli which failed to yield a positive result; the latter is the shortest interval which did yield a positive result. These show that variations in diastolic filling produce no change in the duration of either the A.R.P. or the R.R.P. Fig. 5 shows that the A.R.P. of a heart is the same for two different initial fillings. In this as in all subsequent tracings systole is represented by a downward movement of the recording lever.

TABLE I

Experiment 27/11/28. Rate 24/minute

Filling in cc.	A.R.P.	End of R.R.P.	Pressure cm. of H_2O
0.01	1.00+0.95-	1.39	10
0.03	1.02+0.95-	1.41	50
0.06	1.00+0.97-	1.37	62
0.07	1.01+0.98-	1.39	64

Experiment 21/2/29 Rate 18/minute

0.01	1.24+1.18-	1.79	11
0.03	1.23+1.18-	1.76	37
0.05	L.23+1.19-	1.78	58

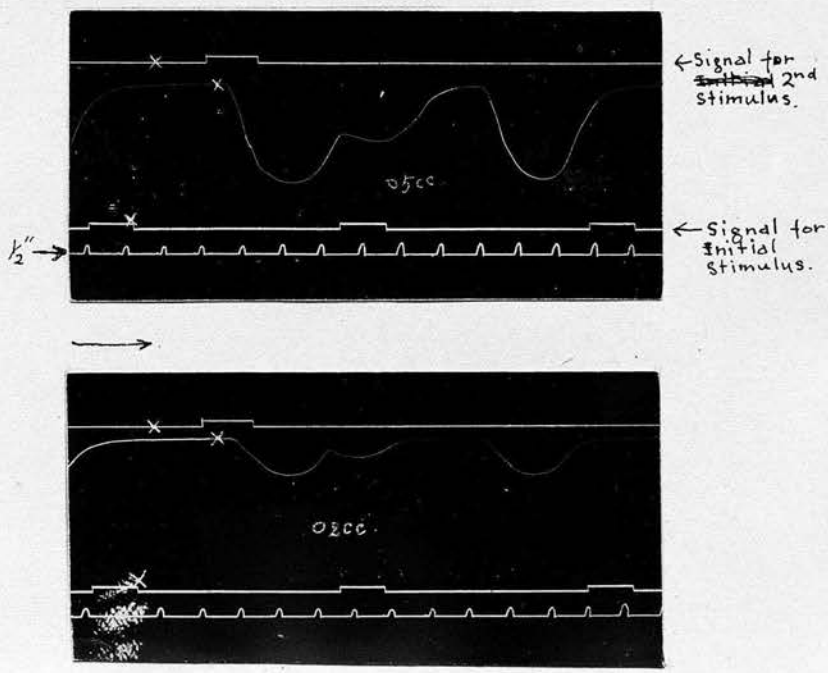


Fig 5.
Corresponding Points are marked with Crosses (⊗).

In other experiments the curves of recovery of excitability at various fillings were plotted. In this case it was more difficult to avoid errors due to the development of the hypodynamic condition. Typical results are shown in Fig. 6 which shows that the course of the curve of recovery in excitability is not altered by variations in the diastolic filling.

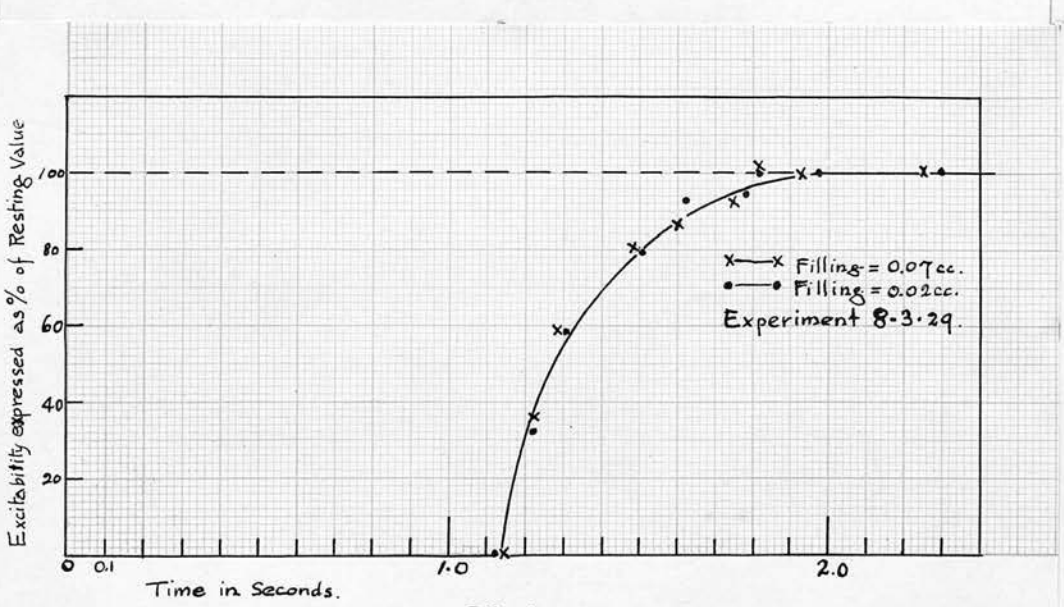


Fig. 6.

(c.) Discussions.

(a.) As has been pointed out before, the extent of

metabolic change resulting from the contraction process can be varied very extensively by simply varying the diastolic filling of the heart. The results of these experiments show, however, that such variations have no effect whatever on the duration of the refractory period. It would thus appear that the duration of the refractory period is independent of the extent of metabolic change associated with contraction. Such a conclusion may have some bearing on the causation of the refractory period.

Two hypotheses have been put forward to explain the occurence of the refractory period, namely the exhaustion hypothesis and the permeability hypothesis (Adrian (15)). According to the former hypothesis, the refractory period is due to the exhaustion of some active material inside the tissue fibres. Immediately after an effective stimulation the store of this material is exhausted, and the tissue in consequence is unable to respond to a second stimulus (A.R.P.). As time passes, however, this material gradually reaccumulates and the excitability gradually returns to normal (R.R.P.).

According to the permeability hypothesis, which was put forward to harmonise many experimental facts with the cell membrane theory of conduction of the propagated excitatory disturbance, excitation depends on the ability of a stimulus to break down the state of semi-permeability of some membrane, while the re-

fractory period depends on the stability of this membrane. Immediately after the passage of a propagated disturbance the membrane becomes unusually stable, so that further break down of semi-permeability i.e. excitation, is impossible (A.R.P.). After a time, however, the membrane gradually returns from a stable to a more unstable condition and as a result, the excitability gradually returns to normal (R.R.P.).

Adrian (loc. cit.) has given good reasons to show that the exhaustion hypothesis is untenable. The results of these experiments provide further evidence against this hypothesis, for were the refractory period due to the exhaustion of some active material, then it would be difficult to understand why an increase in the metabolic breakdown is not followed by a corresponding lengthening of the time during which reaccumulation of this material is supposed to take place. The permeability hypothesis, on the other hand, is quite in keeping with such results, since according to it, the duration of the refractory period depends only on the physical state of some membrane and not at all on the extent of metabolic change which has occurred.

(b) There has been much controversy as to whether or not the excitation process and the contraction process in muscular tissues are separable from each other. According to the conception of Keith Lucas (16), when a muscle responds to a stimulus there are

two separate processes taking place, namely the excitation process and the contraction process. The former process is essentially the same in all excitable tissues, whether nervous or muscular, and is composed of two elements, namely a local excitatory disturbance and a propagated disturbance. It is almost unassociated with heat liberation and is probably mainly physical in nature, although recent work on the oxygen consumption of nerves by Fenn (17) and Gerard (18) seems to show that there is also some chemical change associated with nervous activity. The oxygen comsumption associated with excitation, however, is so small when compared to that associated with the contraction process that it may be considered negligible. The contraction process on the other hand, is present only in muscular tissues in which it is a consequence of the propagated disturbance and not an essential part of it. It is closely associated with lactic acid metabolism and the development of tension. According to this view then, these two processes are distinct and separable from each other.

On the other hand, some authorities consider that these two processes are inseparably associated with each other. In particular, this view has been strongly supported by Fulton (19) who maintains that excitability and contractibility are inseparable and that in muscles the mechanism of production of

electrical response and the mechanism controlling initial heat production are one and the same. It is of interest therefore, to see whether or not the results of these experiments have any bearing on the question and if so, whether they support the former or the latter conception.

Since both the refractory period and the excitation process are dependent on the physical state of some membrane, it may be supposed that they are closely related to each other. Indeed, according to Bramwell and Lucas (20), the refractory period is a direct consequence of the propagated disturbance. It is well known from the observations of Adrian and Keith Lucas (21) that an excitatory disturbance set up during the relative refractory period is of smaller magnitude than the normal, and that the refractory period following this disturbance is of shorter duration than the normal. It may, therefore, be supposed that the duration of the refractory period is in some way related to the magnitude of the propagated disturbance which precedes it. This, in fact, is the view of Bayliss (22) who considers that the duration of the refractory period is dependent on the magnitude of the propagated disturbance. Therefore, through the duration of the refractory period one may indirectly obtain some idea as to the magnitude of the excitation process.

Since variations in the diastolic filling have no influence whatever on the duration of the refractory

period and since the refractory period is so intimately bound up with the excitation process, one may be justified in assuming that similar variations in filling also have no influence on the excitation process. But such variations have a profound influence on the various phenomena which are associated with the contraction process, such as the tension developed, the oxygen consumption, and the heat liberation. Therefore, in variations in the diastolic filling we have a condition in which the contraction process is profoundly affected while at the same time the excitation process is not at all influenced. Such a state of affairs would be quite unaccountable by Fulton's conception, for it is difficult to understand how one process could vary independently of the other if they were inseparable and dependent on the same mechanism. On the other hand, such findings are quite intelligible according to the conception of Keith Lucas. The results of these experiments, therefore, constitute an indirect evidence in support of the view which maintains that excitation and contraction are separable and are opposed to the other view which holds that these two phenomena are inseparable.

B. Effect of Diastolic Filling on the Duration of Mechanical Response in Isochoric Contraction.

This question has been investigated by a number of workers, especially Daly (loc. cit.) and Segall and

Anrep (loc. cit.). Their results in general agreed with each other, and according to the latter observers, the total duration, the period of rising tension, and the period of falling tension were prolonged by increased diastolic filling. They further observed that, for the period of rising tension, the increase was more marked with smaller fillings, reaching a maximum when the heart was filled to $\frac{2}{3}$ its full capacity; while for the period of falling tension, the maximum increase occurred with the larger fillings. These workers divided the duration of mechanical response into the period of rising tension and the period of falling tension. The former was measured from the point where the curve left the base line to the middle of its flattened top and the latter from this point to where the curve returned once more to the base line. In the experiments described below a different method of measuring the duration of mechanical response was employed and the results obtained with it were compared with those of previous workers.

In the case of fresh unfatigued skeletal muscles with intact circulation, Fulton (23) described the fundamental characteristics of the curve of a single isometric twitch as consisting of

(1) an abrupt and convex ascent.
(2) a flat plateau at the summit of the twitch.
(3) an angle at the end of the plateau due to its abrupt termination.

This angle has been termed the "subsidence angle" by Fulton who has adduced good reasons to show that it

represents a fundamental break in the underlying contractile process and consequently, represents the end of active contraction. Relaxation, according to his view, is a purely passive process. He, therefore, measured the duration of the isometric twitch from the beginning of the electrical response to the "angle".

In the case of the frog's heart, Junkmann (14) pointed out that the curve of isochoric contraction of freshly excised ventricles showed a well marked plateau and that this plateau disappeared after a few hours' perfusion. He did not, however, mention the occurrence of the "angle", although in some of his figures an angle could be readily made out. In the course of the experiments described above both the "plateau" and the "angle" were observed in the records of isochoric contraction of freshly excised ventricles. Fig. 7 shows the record of isochoric contraction of a freshly excised frog's ventricle. It will be noticed that both the plateau and a definite angle are present.

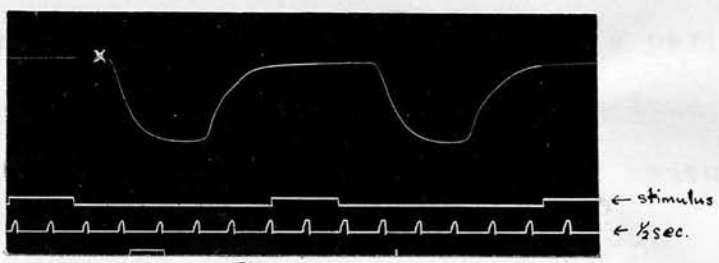


Fig. 7.

Method of Measurement.

In view of Fulton's method for measuring the duration of the isometric twitch, it was considered desirable to measure the duration of mechanical

response of the frog's heart by the following method. In this method, the duration of mechanical response is measured from the stimulus to the "angle" and the term D.M.R. is employed to denote this period in the rest of this thesis. The duration of relaxation is measured from the "angle" to the point at which 90 per cent relaxation has occurred. This method of measuring the period of relaxation is more accurate than the usual method of measuring to the point, at which complete relaxation occurs because the curve of relaxation follows a die-away curve and it is impossible to be sure of the exact moment when complete relaxation occurs.

It will be noticed that the latent period is included in the D.M.R. Recent work on the latent period, however, all tend to show that the so-called latent period observed in ordinary kymographic records is nearly entirely the result of mechanical lag in the recording apparatus. Thus, with every improvement in the sensitivity of the recording apparatus, there is a steady reduction in the duration of this period. According to Fulton (24) the duration of the true latent period of frog's skeletal muscles, as measured by the torsion-wire myograph, is only 1.5-2.00σ. On the other hand, the very existence of the latent period was denied by de Jough (25) who found that by recording the mechanical response of the frog's heart with an apparatus of great sensitivity such as the

'snaarmyograaf', the stimulus and the beginning of mechanical response were simultaneous. It is still a matter of controversy as to the existence of a true latent period but it is clear that if a true latent period exists at all, it must be of such a duration as to be quite undetectable by the Kymographic method of recording which was employed in these experiments. For this reason, the latent period was ignored altogether and the mechanical response was considered to begin simultaneously with the stimulus.

The tracings were measured directly with the aid of a reading microscope and the results also were checked by measuring enlargements of records taken on smoked celluloid films.

Owing to the circular movement of the recording lever, due corrections were made for any deviation of the writing point from the vertical, especially when the deflection of the lever was great as in the case of large initial fillings when the heart developed a large amount of pressure.

Results.

Nine experiments were performed and the results of two typical ones are given in Table II. Fig. 8 shows the actual tracings from which the first group of data in Table II was obtained by direct measurement. In these tracings the signal for the stimulus was somewhat to the left of the writing point of the lever and this should be corrected.

when calculating the time interval.

TABLE II

Experiment 22/1/29. Rate 9/minute

Filling in cc.	Stimulus to "Angle" or D.M.R. in seconds	"Latent Period" in seconds	D.M.R. minus "L. P." in secds.	"Angle" to 9/10 Relaxation in seconds	Pressure added by contraction cm. of H_2O
0.01	2.00	0.36	1.64	0.75	39
0.02	1.97	0.29	1.68	0.88	63
0.03	1.95	0.20	1.75	0.90	85
0.05	1.94	0.16	1.78	1.37	93
0.06	1.94	0.06	1.88	1.37	92

Experiment 9/2/29. Rate 10/minute

0.01	1.73	0.20	1.53	0.62	35
0.02	1.73	0.16	1.57	0.71	52
0.03	1.68	0.14	1.54	0.85	64
0.04	1.66	0.12	1.54	0.92	74
0.05	1.70	0.11	1.59	0.97	78
0.06	1.71	0.11	1.60	1.10	83
0.08	1.67	0.07	1.60	1.30	85

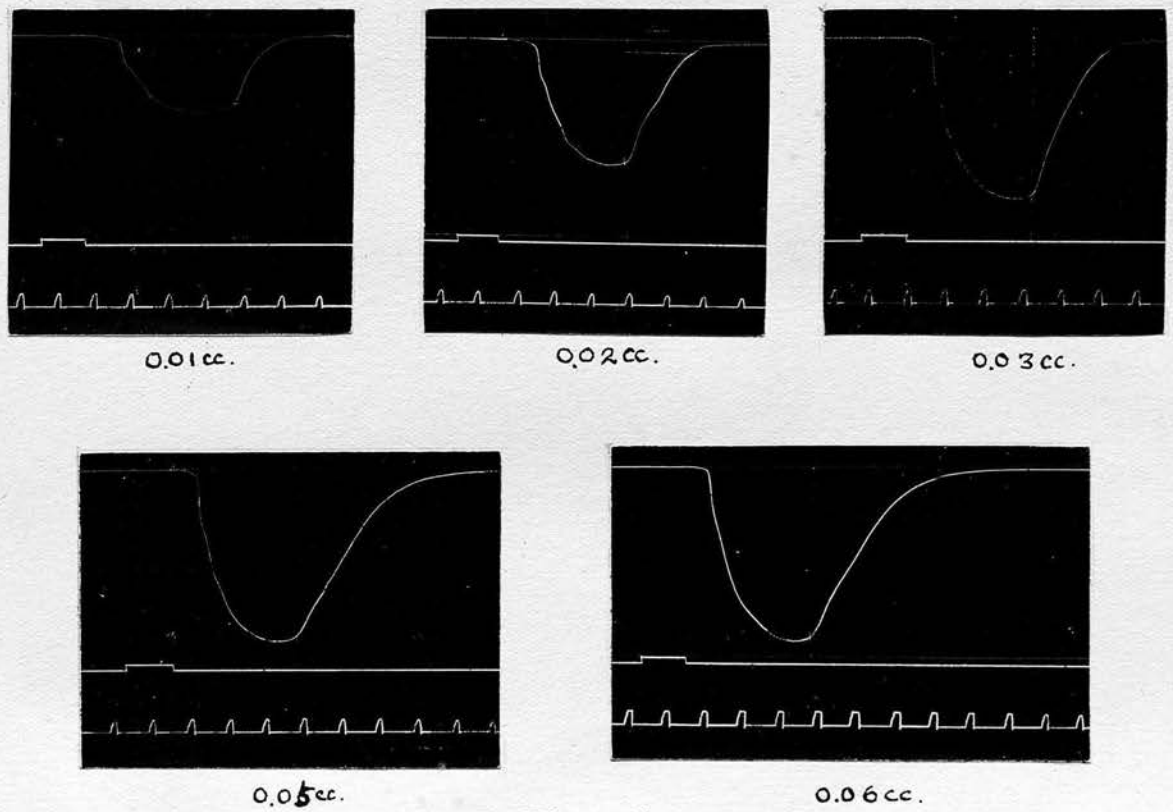


Fig. 8.

It will be seen from this table that the D.M.R. for various diastolic fillings agree closely with each other, any difference between them being less than 0.1" which is within the limits of error of the method. The average of the experiments shows, however, a slight shortening of the D.M.R. when the diastolic filling is large. These results, therefore, show that increase in filling certainly does not increase the D.M.R. but either causes no change or else a slight shortening. In connection with this last point, it may be mentioned that Drs. Bogue and Mendez, employing optical recording apparatus, have found a definite shortening of the D.M.R. of the frog's heart when the filling is increased. Their results have not yet been published but they have kindly permitted me to refer to them.

In order to show the difference that might result if the so-called latent period were not included in the D.M.R., a few of the tracings were measured from the point where the curve left the base line to the "angle". The results thus obtained are given in column 4 of Table II. It will be observed that they agree with the results of previous workers in that increased filling increases the duration of mechanical response. The progressive shortening of the "latent period" or rather the mechanical lag, which accompanies increments of diastolic filling, is clearly shown in Column 3 of the same Table.

The duration of relaxation increases as the diastolic filling is increased, as shown in Table II. This result is in accordance with the results of previous workers.

Discussion.

The method employed in these experiments for measuring the D.M.R., namely measurement from stimulus to the "angle", differs from previous measurements of the period in two respects, firstly, in that the "latent period" in included in the D.M.R. and secondly, in that the angle is taken as the end of the measurement instead of the summit of contraction.

As regards the first point, it has been pointed out previously that the observed "latent period" is almost entirely due to instrumental lag, and that the true latent period is of such a short duration as to be undetectable by the apparatus employed in these experiments. Furthermore, my measurements show that the so-called latent period decreases when the filling of the heart increases, and that this variation is sufficient to produce a considerable error. The conclusion of previous workers that increased filling increases the duration of mechanical response can be accounted for by the error due to variations in mechanical lag. When this error is eliminated, by including the observed "latent "period" in the D.M.R., then the duration of mechanical response is found to be almost unaltered

by alterations in the diastolic filling.

The selection, as end point of mechanical response, of the "angle" instead of the summit of contraction has two advantages; firstly, the former is a much more definite point to measure and secondly the angle, according to Fulton, represents the true end of the contraction process. If this view be accepted, then the duration of mechanical response, as measured from the stimulus to the "angle", is a true measurement of the duration of the contraction process, except that a few sigma should be subtracted to allow for the true latent period. This statement, however, is true only for measurements made from records obtained by sensitive apparatus. For measurements obtained from kymographic records, further subtraction must be made to allow for the mechanical lag which intervenes between the actual end of the contraction process and the occurrence of the "angle" on the tracings.

Since the mechanical lag shortens with increased filling, the shortening of the D.M.R. noted above when the filling is large may be due to a shortening of this mechanical lag. A comparison of the extent of shortening of the "latent period" and of the D.M.R. at various fillings (see Table II) shows that the shortening is greater in the former case than in the latter case. This, however, is not an argument

against the latter being due to variations in the mechanical lag, for the amount of mechanical lag in the recording apparatus at the beginning of contraction and at the very onset of relaxation are different. A rubber manometer was used in these experiments and the greater part of the mechanical lag of the recording apparatus occurred here. In such a manometer the mechanical lag varies inversely with the degree of stretching of the rubber membrane. At the beginning of contraction the rubber membrane is only stretched by a small force equal to the diastolic pressure, whereas at the commencement of relaxation it is stretched by a much greater force equal to the systolic pressure. This difference in the degree of stretching of the manometer will result in a difference in the mechanical lag at the beginning and at the end of contraction, which may account for the difference in the extent of shortening of the "latent period" and of the D.M.R. at various initial fillings.

The results obtained by this new method of measurement show, therefore, that the duration of active contraction, like the refractory period, is not altered by altering the diastolic filling and thereby altering the amount of metabolic change associated with contraction.

II. <u>The Effect of Various Ions on the Duration of the Refractory Period and on the Mechanical Response.</u>

The effect of various ions on the duration of the refractory period has been investigated by several workers with results that are not in general agreement. For this reason it has been considered desirable to further investigate this question.

Method.

The apparatus and the technique employed in these experiments were the same as those employed in previous experiments. The perfusing fluid containing the necessary ions was placed in the upper canula while only saline was placed in the lower canula. In every case the heart was perfused for at least 5-10 minutes with the fluid before readings were taken. Only the absolute refractory period (A.R.P.) was determined because it was found that when fluids containing abnormal proportions of the different ions were used, the threshold excitability changed progressively as the experiment went on, so that the results obtained for the relative refractory period were unreliable.

The various perfusing fluids employed in these experiments were made according to the following formulae:-

	Normal	4xCa.	¼Ca.	4xK	¼K	Acid	Alkaline
NaCl.	0.65%	0.65%	0.65%	0.65%	0.65%	0.65%	0.65%
KCl.	0.015%	0.015%	0.015%	0.06%	0.004%	0.015%	0.015%
$CaCl_2$ (anhyd)	0.011%	0.044%	0.003%	0.011%	0.011%	0.011%	0.011%
Sod. Phosph.	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%
pH.	7.5	7.5	7.5	7.5	7.5	6.2	8.2

The reaction of the solutions was varied by adding N/10HCl. or N/10NaOH and the pH was determined by the calorimetric method.

Results.

Ten experiments were performed and the typical results of one such experiment are given in Table III.

TABLE III

Experiment 16/3/29 Filling 0.04cc. Rate 18/minute

Time	11:15	11;30	11:35	11:43	11:50	12:03
Ion	Normal	4xCa	Normal	¼Ca	Normal	4xK
A.R.P. in secds.	1.30− 1.34+	1.08− 1.15+	1.30− 1.34+	1.38− 1.43+	1.31− 1.36+	1.82− 1.88+
D.M.R. in secds	1.48	1.35	1.48	1.74	1.50	1.44
Pressure added in cm. H_2O	74	79	63	13	60	45
Time	12:15	12:35	12:40	12:45	12:50	12:57
Ion	Normal	¼K	Normal	pH 8.2	Normal	pH 6.2
A.R.P. in seconds	1.30− 1.36+	1.50− 1.61+	1.20− 1.29+	1.20− 1.27+	1.23− 1.29+	1.34− 1.40+
D.M.R. in seconds	1.44	1.50	1.39	1.40	1.40	1.54
Pressure added in cm. of H_2O	46	46	45	40	40	22

In general the effect of various ions may be summarised as follows:-

(1) Calcium excess (4xCa).

Calcium excess shortens both the A.R.P. and the D.M.R. equally. The pressure added by contraction in slightly increased. The form of the mechanogram is not altered (Fig. 9).

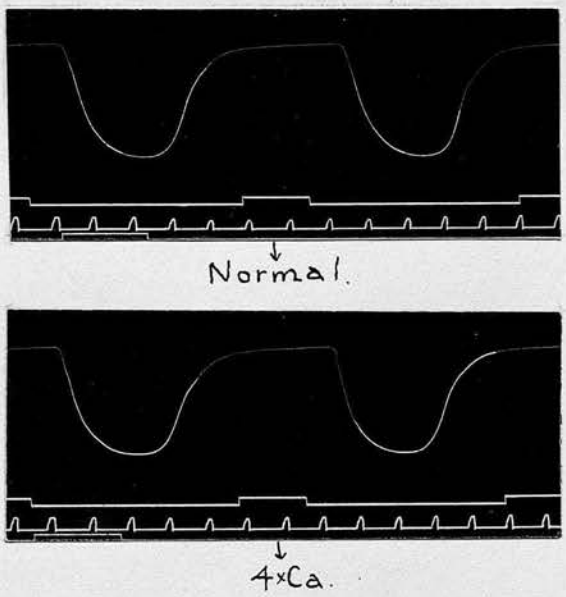


Fig. 9.

(2) Calcium lack ($\frac{1}{4}$Ca).

Calcium lack prolongs both the A.R.P. and the D.M.R. The pressure added by contraction is greatly and rapidly reduced. The form of the mechanogram (Fig. 10) is not altered, being in general quite like that of a normal heart developing

the same amount of pressure.

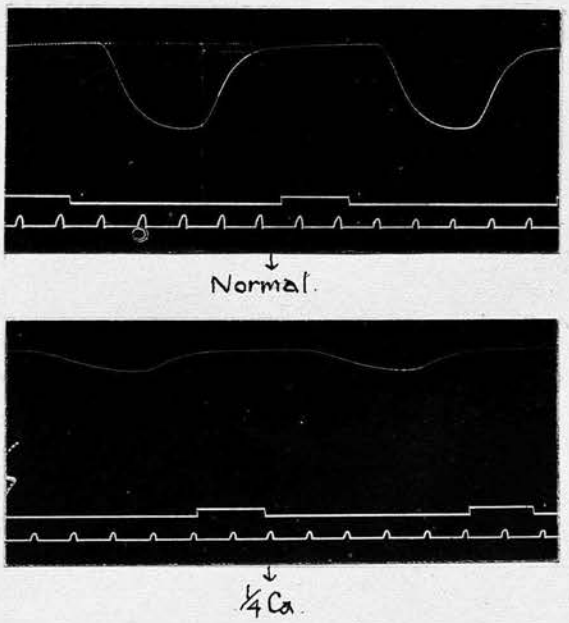


Fig. 10.

(3) Potassium excess (4xK).

Potassium excess prolongs the A.R.P. but shortens the D.M.R. The pressure added by contraction is much reduced. The form of the mechanogram is rendered very abnormal (Fig. 11). Instead of the usual convex ascent, the plateau, and the "angle", it consists of a short ascent and then a short fall with no sign of the plateau or of the "angle".

The "latent period" is much prolonged. The absence of the "angle" renders accurate measurement of the D.M.R. difficult. Owing to this difficulty and to the abnormality in the form of the mechanogram, the measurements obtained for the D.M.R. must be accepted with due reserve.

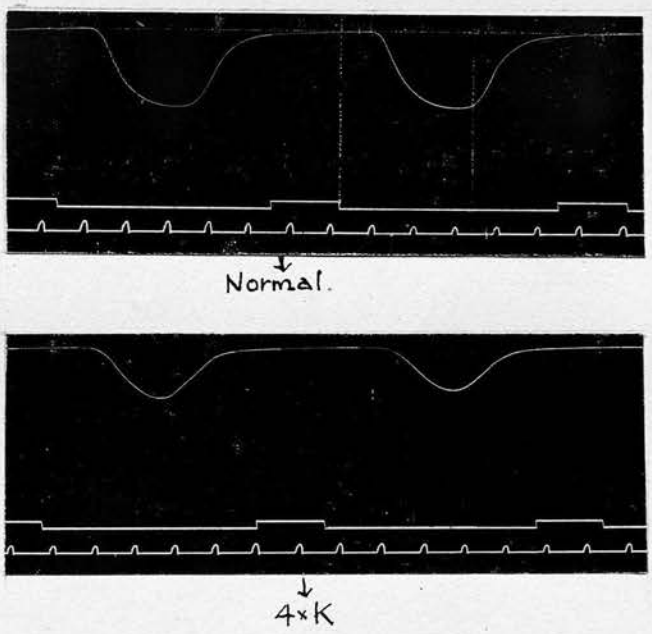
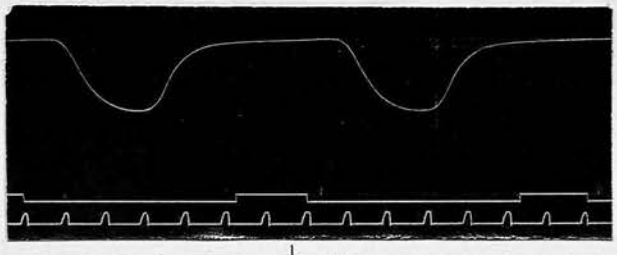


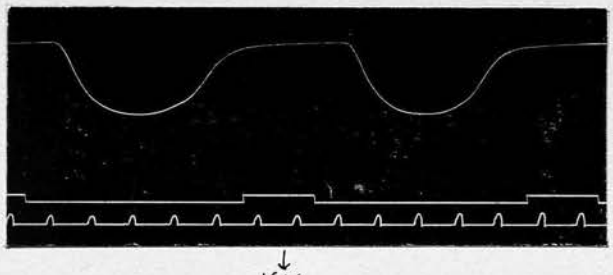
Fig. 11

(4) Potassium lack ($\frac{1}{4}$K).

Potassium lack prolongs the A.R.P. and the D.M.R. The pressure added by contraction is not altered. The mechanogram also undergoes some alteration (Fig. 12). The "angle" becomes obscured while relaxation is much prolonged.



Normal.



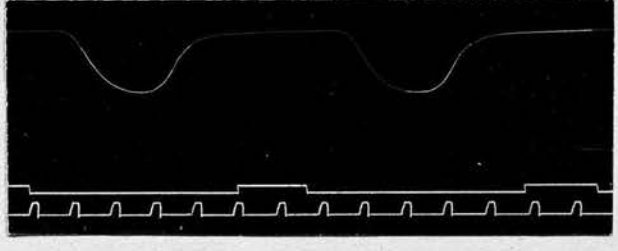
¼K.

Fig. 12.

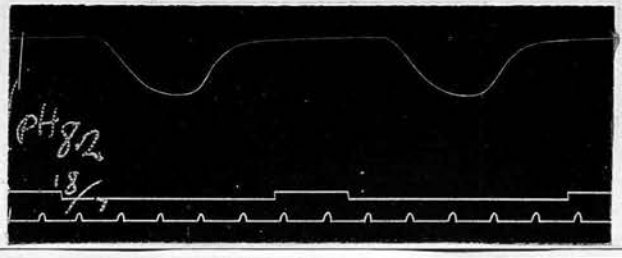
(5) Alkalinity (pH8.2).

A moderate degree of alkalinity (pH8.2) has no effect either on the A.R.P. or on the D.M.R. The pressure added by contraction is slightly, if at all, reduced. The form of the mechanogram is not altered (Fig.13). A greater degree of alkalinity was not investigated because when the pH was above 8.2 the phosphate was precipitated from the fluid.

Fig. 13.



Normal.



pH 8.2

(6) Acidity (pH6.2).

Acidity prolongs both the A.R.P. and the D.M.R. The pressure added by contraction is greatly reduced, The general form of the mechanogram is not much altered (Fig. 14).

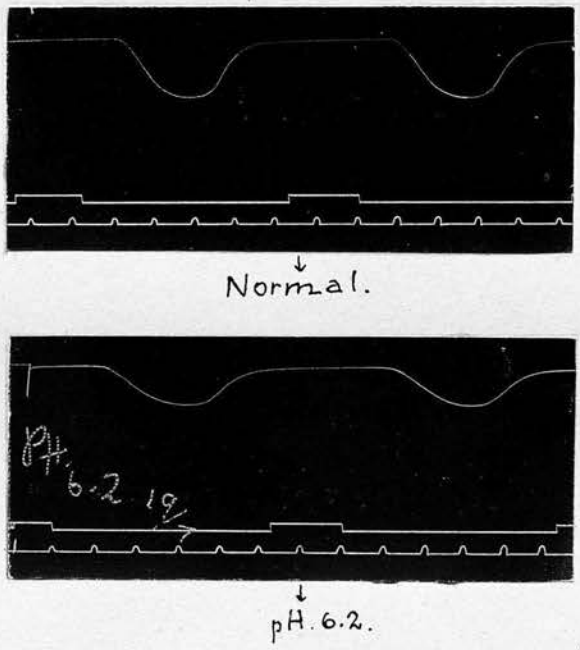


Fig. 14.

Results of other workers.

As mentioned previously, the results obtained by previous workers were not in general agreement with each other. They are summarised in Table IV

TABLE IV

Ion	Ca excess		K excess	
Effect on Refractory Period	R.P.+	R.P.−	R.P.+	R.P.−
Name of Authors & Reference		Walther (26) Denning (27) Boehm (28) Trendelenburg (29) Schütz (30)	Ringer and Sainsbury (31) Denning (27) Trendelenburg (29) Schütz (after prolonged perfusion) (30)	Schütz (at onset of action) (30)

Ion	Acidity		Alkalinity	
Effect on Refractory Period.	R.P.+	R.P.−	R.P.+	R.P.−
Name of Author and Reference.	Beritoff & Tskimanuri (after ~~short~~ prolonged perfusion) (32) Carter and Dieudaide (dog's heart) (12)	Beritoff & Tskimanuri (after ~~prolonged~~ short perfusion) (32)		Carter and Dieudaide (dog's heart) (12)

It is clear from this table that there is general

agreement only as regards the effect of Calcium excess in shortening the duration of the A.R.P. It is difficult to account for the difference in the results obtained by different workers but as has been shown in a previous section, experimental errors are easily introduced in experiments such as these. It is possible that some of the differences might be due to experimental errors arising out of neglecting such important points as,for example, the proper placing of the electrodes or the special measures taken to guard against the peculiar effect described by Drury and Love which might very well be brought about by abnormal proportions of various ions. Unfortunately, however, these points were not specially mentioned in most of the reports, so that one cannot be quite sure on this point. In view of this uncertainty, only the results of my experiments are taken into consideration in drawing the conclusions of this thesis.

Discussion.

A glance at the results of these experiments will show that there is absolutely no relationship between the duration of the refractory period and the amount of pressure added by contraction. Thus, an almost normal amount of added pressure may be associated with either a shortening of the refractory period, as in the case of calcium excess or a lengthening of the refractory period, as in the case of potassium lack, while a reduced amount of added pressure may

even be associated with a lengthening of the refractory period, as in the case of calcium lack and also in the case of acidity. Since the development of tension (or pressure in the case of the heart) is an essential part of the contraction process and since the duration of the refractory period may be considered an index of the magnitude of the propagated excitatory disturbance, this lack of relationship between the refractory period and the added pressure again demonstrates the possibility of varying one process independently of the other. As has been explained before, such independent variation points to the unlikelihood of these two processes being inseparably associated with each other. Therefore, in these experiments we have further support for the view which maintains the separability of excitation and contraction.

III. The Effect of Rate of Stimulation on the Durations of the Refractory Period and of the Mechanical Response.

Only a few experiments were performed to study the effect of rate of stimulation on the duration of the refractory period, as this is already well known from the work of Trendelenburg (33) and Mines (34) on the frog's heart and of Lewis and his co-workers (35) on the mammalian heart. The experiments described here were performed mainly to confirm the results of previous workers.

The apparatus and the technique for determining the duration of the refractory period and of the mechanical response were the same as those employed in previous experiments. The rate of stimulation was varied by varying the rate of rotation of the contact breaker.

Results:

The results of these experiments (five in number) confirm the results of previous workers in that increased rate of stimulation shortens both the A.R.P. and the R.R.P. This is shown in Fig. 15 which represents the curve of recovery of excitability of a heart under two different rates of stimulation. A few experiments were also performed in which only the A.R.P. and the D.M.R. were measured for various rates of stimulation. Typical results are given in Table V which shows the A.R.P. and the D.M.R. to be affected similarly by variations in the rate of stimulation. The tracings from one of these experiments are shown in Fig. 16.

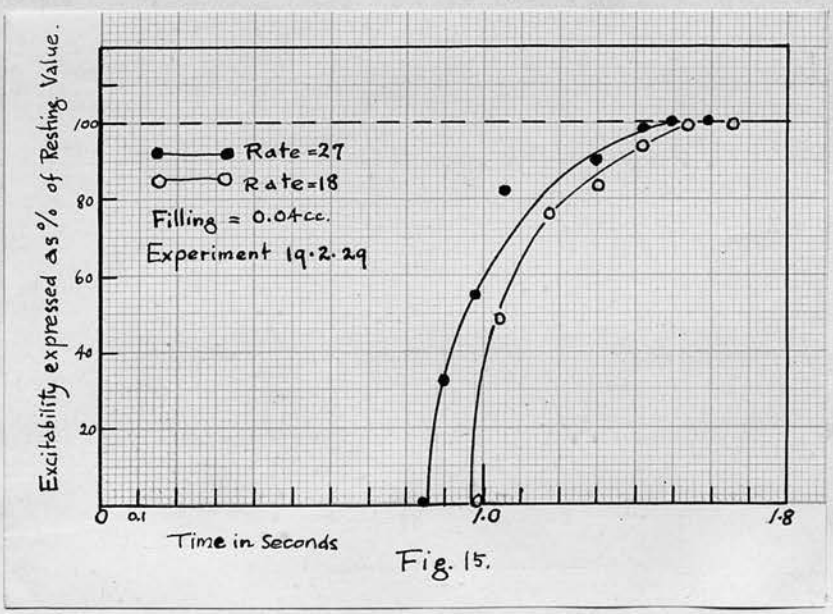


Fig. 15.

TABLE V

Experiment 19/3/29 Filling 0.04cc.

Rate of Stimulation	A.R.P. in seconds	D.M.R. in seconds
28	1.02 – 1.06 +	1.18
18	1.30 – 1.36 +	1.40
10	1.38 – 1.51 +	1.56

Experiment 20/3/29 Filling 0.05cc.

27	0.87 – 0.94 +	1.04
18	1.00 – 1.10 +	1.17
10	1.11 – 1.25 +	1.29

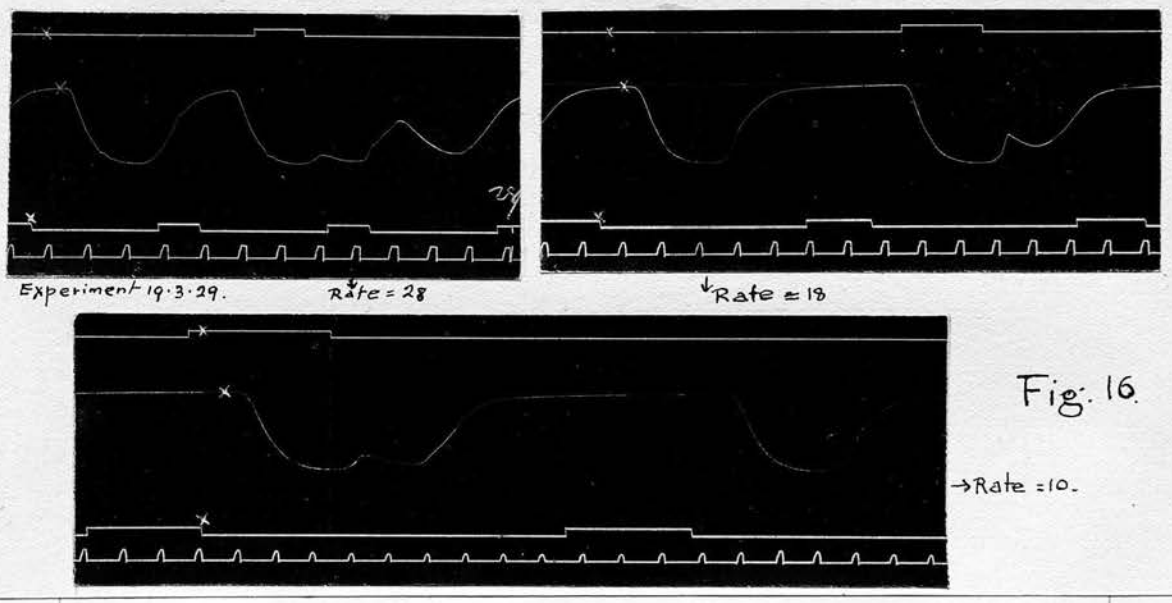


Fig. 16.

It may be noted that when the rate of stimulation is comparatively great, i.e. 27 per minute, the "angle" in the curve of isochoric contraction tends to become

rather obscured. Since in these experiments the faster rate was always investigated before the slower ones, the tendency for the "angle" to become obscured could not be due to deterioration of the preparation but must be due to the effect of increased rate of stimulation. In the case of frog's skeletal muscles Fulton pointed out that similar obscuring of the "angle" occurred with excessive stimulation.

Discussion.

It has been shown by Weizsäcker (36) that within certain limits, i.e. 30 per minute, frequency of stimulation has no influence on the oxygen consumption per beat of the heart. This observation has quite recently been confirmed by Clark and White (6). Since oxygen consumption may in a large measure be regarded as an index of the extent of metabolic change occurring in the heart as a result of contraction, the above observation would mean that, within wide limits, rate of stimulation has no influence on the extent of metabolic change associated with individual heart beats. Yet rate has a profound influence on the duration of the refractory period, as the above experiments show. Here there is a condition which, within wide limits, affects the refractory period without at the same time affecting the extent of the metabolic change of the heart. In other words, it is the exact reverse

of variations in filling. By applying the same arguments as before, one again finds support for the view which maintains that excitation and contraction are separable.

IV. The Relation of the Refractory Period to other Phenomena associated with Contraction.

(1) The extent of Metabolic change.

It has been repeatedly shown in previous pages that the duration of the refractory period is absolutely unrelated to the extent of the metabolic change associated with contraction. Therefore, it is only necessary here to summarise in tabulated form the various conditions which influence the two phenomena differently, so as to emphasise the lack of relationship between them. Such a summary is shown in Table VI in which this lack of relationship is clearly ~~shown~~ demonstrated.

TABLE VI

Condition	Extent of Metabolic change	Duration of Refractory Period
Filling	increased	no change
Rate	no change if rate is less than 30 per minute	diminished
Ca excess	slinghtly increased	diminished
Ca lack	much diminished	increased
K excess	diminished	increased
K lack	no change	increased
Acidity	much diminished	increased
Alkalinity (pH 8.2)	slightly diminished	no change
Temperature	increased (36)	diminished (27)(41)(42)

(2). The Duration of Mechanical Response.

It is generally considered by physiologists that there is a close relationship between the length of systole and the duration of the refractory period. In particular, Schultz (37) considered that the relation between the length of systole and the duration of the refractory period is a constant one. On the other hand, Dennig(27) as a result of studying the effects of cold and certain ions on the frog's heart came to the conclusion that the refractory period and the mechanical response did not vary identically. It must be remembered, however, that these workers employed the usual method of measuring the duration of mechanical response which yielded unreliable results, as has been shown previously. It is desirable, therefore, to reconsider this question in the light of the results obtained by the new method of measuring the duration of mechanical response.

In practically all the experiments performed in this research it was noticed that the absolute refractory period and the D.M.R. always varied

together, as shown in Table VII.

TABLE VII

Condition	D.M.R.	A.R.P.
Filling	no change or ?slight shortening	no change
Rate	shortened	shortened
Hypodynamic State	shortened	shortened
Ca excess	shortened	shortened
Ca lack	prolonged	prolonged
K excess	shortened	prolonged
K lack	prolonged	prolonged
Acidity	prolonged	prolonged
Alkalinity pH 8.2	no change	no change
Temperature	shortened (43)	shortened

It will be seen from Table VII that with the exception of perfusion with excess of potassium, the absolute refractory period and the D.M.R. always vary together. In the case of potassium excess, it has been pointed out previously that owing to the

abnormality of the mechanogram, accurate measurement of the D.M.R. was impossible and that the results obtained were unreliable. Hence, no weight should be attached to this discrepency between the duration of the absolute refractory period and the duration of the mechanical response. Judging from the results of the above experiments one can, therefore, say that the absolute refractory period and the duration of mechanical response always vary together.

Moreover, it was found impossible to cause a second contraction to occur before the "angle" of a previous contraction, although in some cases the second contraction occurred very close to it. This suggests that the "angle" coincides with the limits of the absolute refractory period. When one compares the duration of the absolute refractory period and the duration of the mechanical response however, one invariably finds that the former is shorter than the latter. This is shown in Table VIII.

TABLE VIII

Date of Experiment	D.M.R. in seconds	A.R.P.* in seconds	Difference bt. D.M.R. & A.R.P.	"Latent Period"
16/3/29 Rate 18/minute	1.48	1.32	0.16	0.18
5/3/29 Rate 18/minute	0.95	0.86	0.09	0.16
19/3/29 Rate 18/minute	1.40	1.33	0.07	0.08
19/3/29 Rate 10/minute	1.56	1.44	0.12	0.14
19/3/29 Rate 28 /minute	1.18	1.04	0.14	0.08
14/3/29 Rate 18/minute	1.35	1.24	0.11	0.09
18/3/29 Rate 18/minute	1.17	1.05	0.12	0.07
18/3/29 Rate 10/minute	1.29	1.18	0.11	0.12
18/2/29 Rate 27/minute	1.04	0.91	0.13	0.18

*The A.R.P. given here is the averge of the longest time interval between stimuli which failed to yield a positive result and the shortest interval which did yield a positive result.

It must be remembered, however, that owing to a mechanical lag, the "angle" in these tracings did not occur simultaneously with the end of the contraction process, but occurred after an interval corresponding to the mechanical lag. A comparison of the "latent period" or mechanical lag and the difference between the D.M.R. and the A.R.P. (table VIII) shows that this difference between the D.M.R. and the A.R.P. is within the limits of the mechanical lag and that in most cases there is a close agreement between them. This suggests that the difference noted above can be accounted for entirely by mechanical lag in the recording apparatus. There is, therefore, good reason to think that the end of active contraction coincides with the end of the refractory period and in mechanograms obtained with sensitive apparatus this would be at the "angle".

(3). The Duration of Electrical Response.

The relation of the refractory period to the duration of the action current has not been investigated in this series of experiments. Therefore, only the results of other workers are considered here.

Early workers on this question such as Burdon-Sanderson and Page (38) and Tait (39) considered that the total refractory phase of the heart and its action current were roughly of the same duration. Tait further put forward his well known hypothesis that the absolute refractory period corresponds to the rising phase of the action current while the relative refractory period corresponds to the/

declining phase. Later researches by Trendelenburgh (40), however, showed that the total refractory phase of the heart lasted considerably longer than the action current and that the absolute refractory period ended before the end of the action current. Most of the results of these early workers, however, were concerned with diphasic action current which gives very uncertain information as to the rate of decline of the electric change at any particular point in the heart muscle. The most comprehensive account on the relation of the refractory period to the monophasic action current in various excitable tissues was given by Adrian (15). According to this worker, the end of absolute refractory period in the frog's heart coincides with the end of monophasic action current; so that Tait's hypothesis does not hold in the case of heart muscle. The most recent work on the subject by Schütz (30) gave further confirmation to Adrian's conclusion regarding the relation of the refractory period and the duration of monophasic action current in heart muscles. There is, therefore, quite a body of evidence to show that in the case of heart muscle, the ends of the absolute refractory period and of the monophasic action current are the same.

4. Inter-relationship among the Refractory Period, the Duration of Mechanical Response, and the Duration of Electrical Response.

From what has been said in previous paragraphs, it is clear that there is a close inter-relationship among the absolute refractory period, the duration of mechanical response, and the duration of electrical response. It will be remembered that both the end of active contraction and the end of the monophasic action current have been considered to coincide with the end of the absolute refractory period. Therefore, the durations of mechanical response and of electrical response should be the same. This, in fact, is exactly what has been found to be the case by Drs. Bogue and Mendez who have kindly allowed me to quote their results before they are published. According to these workers, the end of monophasic action current of the frog's ventricle always occurs at the "angle" in the mechanogram, as shown in Fig.17 which is reproduced here through their courtesy.

Fig. 17.

A = Monophasic Action Current.
M = Mechanogram.

The results of these workers, therfore, confirm in an indirect manner the conclusions already reached regarding the relations of the absolute refractory. period to the duration of mechanical response and also to the duration of electrical response. Diagrammatically, these relationships can be represented as in Fig. 18.

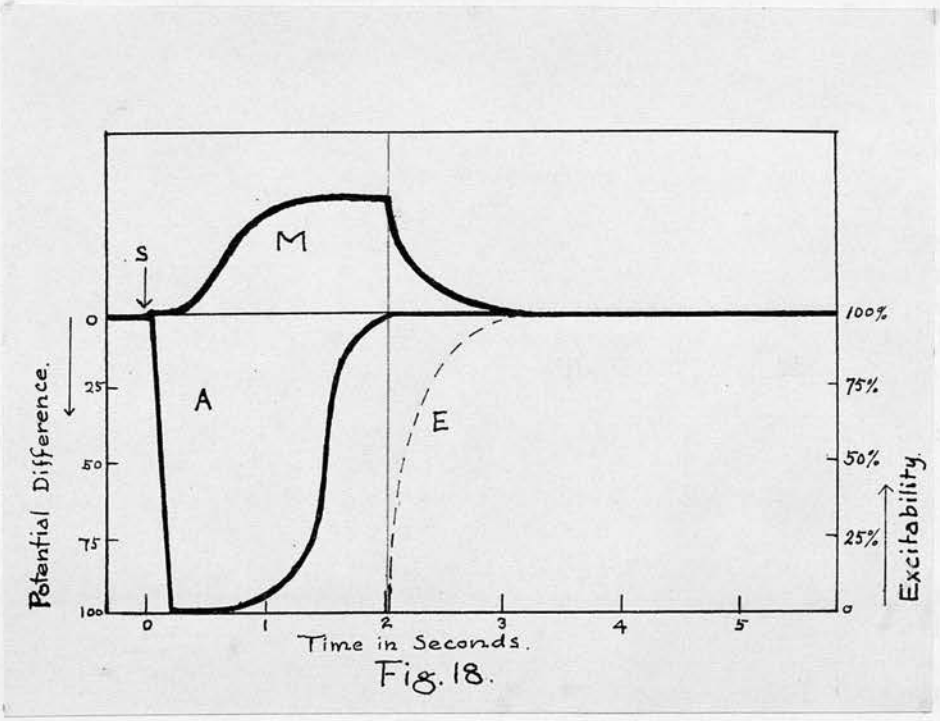


Fig. 18.

M = Machanogram.
A = Monophasic Action Current.
E = Excitability.
S = Stimulus.

V. Summary and Conclusions.

The refractory period and the duration of mechanical response of the isolated frog's ventricle have been investigated under various experimental conditions, with the following results:-

(1) Variations in filling have no influence on the duration of the refractory period. They also have no influence on the duration of mechanical response.

(2) Calcium excess shortens both the absolute refractory period and the duration of mechanical response.

(3) Calcium lack, potassium lack, and acidity all prolong the absolute refractory period and the duration of mechanical response.

(4) Potassium excess prolongs the absolute refractory period. It alters the mechanogram profoundly, so that reliable results as to the duration of mechanical response cannot be obtained.

(5) Moderate degree of alkalinity (pH 8.2) has no influence on either the absolute refractory period or the duration of mechanical response.

(6) Increased frequency of stimulation shortens both the absolute refractory period and the relative refractory period. It also shortens the duration of mechanical response.

(7) Hypodynamic state shortens both the duration of the refractory period and of the mechanical response.

The occurrence of the "plateau" and the "subsidence angle" in the curve of isochoric contraction has been noted in records obtained with freshly excised frog's ventricles.

A new method of measuring the duration of mechanical response of the heart is described. In this method the duration of active contraction is

measured from the stimulus to the "subsidence angle" and the duration of relaxation is measured from the "subsidence angle" to the point at which 90 per cent relaxation has occurred.

The conclusions that have been reached from a consideration of the above results and the results of other workers are as follows:-

(1) The duration of the refractory period is independent of the extent of metabolic change associated with a previous contraction. This conclusion supports the permeability hypothesis of refractory period and is opposed to the exhaustion hypothesis. It also shows indirectly that the excitation process and the contraction process are distinct and separable from each other.

(2) Under normal conditions, the ends of the absolute refractory period, of the mechanical response and of the electrical response of the isolated frog's ventricle are the same, and in the mechanogram, they are represented by the "subsidence angle".

I wish to acknowledge my deep indebtedness to Prof. A.J. Clark, at whose suggestion this work was done, for affording various facilities in connection with the execution of this work and for his constant help and guidance.

The expenses of this work were defrayed by a grant from the Earl of Moray Research Fund for which I wish to express my thanks to the Authorities of the University.

REFERENCES.

1. Frank. Zeit f. Biol. 37, P. 511, 1890.
2. Kozawa. Journ. of Physiol. 49, P. 233, 1915.
3. Daly. Proc. Roy Soc. B.95, P. 279, 1923.
4. Segall and Anrep. Heart 13, P. 61, 1926,
5. Starling and Visscher. Journ. of Physiol. 62, P. 244, 1927.
6. Clark and White. Journ. of Physiol. 66, P. 186, 1928.
7. Fischer. Pflüger's Arch. 216, P. 123, 1927.
8. Clark. Journ. of Physiol. 64, P.123, 1927.
9. Drury and Love. Heart 13, P. 77, 1926.
10. Hermann and Umrath. Pflüger's Arch. 215, P. 365, 1927.

10a. Kupelweiser, Pflüger's Arch., 208, P. 487, 1925.

11. Boesch. Zeit of Biol. 70, P. 371, 1920.
12. Carter and Dieudaide. Bull. of Johns Hopkins Hospital, 39, 99, 1926.
13. Clark. Journ. of Physiol. 47, P. 66, 1913.
14. Junkmann. Arch. f. Exp. Pharmacol. u. Path. 108, P.148, 1925.
15. Adrian. Journ. of Physiol. 55, P. 216, 1921.
16. Keith Lucas. Proc. Roy. Soc. B. 85, P. 495, 1912.
17. Fenn. American Journal of Physiol. 81, P.476, 1927.
18. Gerard. Amer. Journ. of Physiol. 82, P.381, 1927.
19. Fulton. "Muscular Contraction and Reflex Con-"trol of Movement". 1926 P. 246.

~~P. 246~~.

20. Bramwell and Lucas. Journ. of Physiol. 42, P. 495 1911.

21. Adrian and Lucas. Journ. of Physiol., 44 P.93 1912.

22. Bayliss. "Principles of General Physiology." 4th Ed. 1924. P. 390.

23. Fulton. loc. cit. P. 105-126.

24. Fulton. loc. cit. P. 104.

25. de Jough (Quoted from Fulton) "De tijdsverhoudingen tieschen electro - en mechanocardiogram" Leiden, 1923.

26. Walther. Pflüger's Arch. 78, P. 597, 1899.

27. Dennig. Zeit f. Biol. 72, P. 187, 1920.

28. Boehm. Arch. f. Exp. P. u P. 75, P. 230, 1913-14.

29. Trendelenburgh. Arch. f. (Anat. u.) Physiol. P. 283, 1903.

30. Schütz. Zeit. f. Biol. 87, P. 219, 1928.

31. Ringer and Sainsbury. Journ. of Physiol. 4, 350, 1883.

32. Beritoff and Tskimanuri. Zeit f. Biol. 82, P. 213, 1925.

33. Trendelenburgh. Arch. f. Anat. u. Physiol. 1903, (Physiol. Abth.) P. 285.

34. Mines. Journ. of Physiol. 46, P. 349, 1913.

35. Lewis, and Drury, and Bulger. Heart 8, P.83-140, 1921.

36. Weizsäcker. Pflüger's Arch. 148, P. 535, 1912.

37. Schultz. Amer. Journ. Physiol. 22, P. 133, 1908.

38. Burdon-Sanderson and Page. Journ. Physiol. 2, P. 384, 1880.

39. Tait. Quart. Journ. Exp. Physiol. 3, P.221, 1910

40. Trendelenburg. Pflüg. Arch. 141, P. 378, 1911.

41. " " " 144, P. 39, 1912.

42. Eckstein. Pflüger's Arch. 183, P. 40, 1920.

43. Daly and Clark. Journ. of Physiol. 54, P. 275, 1920.